

## New or Noteworthy Plant Collections from Myanmar (8) *Lemna trisulca* (Araceae), *Blyxa aubertii* var. *echinosperma*, and *Najas tenuis* (Hydrocharitaceae)

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During floristic research in Myanmar, some noteworthy aquatic monocots new to the flora of Myanmar were collected: *Lemna trisulca* L. (Araceae), *Blyxa aubertii* var. *echinosperma* (C.B. Clarke) C.D.K. Cook & Lüönd, and *Najas tenuis* A. Braun ex Magnus (Hydrocharitaceae). To compare them with related taxa and/or other regional materials, chloroplast DNA was sequenced for all taxa and the chromosome of *N. tenuis* were counted. Distribution ranges are given for each taxon based on a literature review.

Key words: aquatic plants, *Blyxa*, *Lemna*, Myanmar, *Najas*

With its wealth of plant diversity, Myanmar (Burma) constitutes a significant component of the Indo-Malaysian biodiversity hotspot in both land area and species richness (van Dijk *et al.* 2004). Yet, for a number of reasons, it is one of the most poorly investigated floristic areas in the world, with only five specimens per 100 km<sup>2</sup> (Campbell & Hammond 1989). The reported total of some 11,800 species (Kress *et al.* 2003) may therefore represent only a fraction of the entire flora of the country.

While revising the flora of Myanmar, a decade-long inventory of the plants of Myanmar led by Japanese botanists have discovered many new species, and recorded noteworthy plant collections (Tanaka 2005, 2012a, 2012b, Tanaka *et al.* 2006a, 2006b, 2007, 2009, 2010a, 2010b, 2010c, 2011, Tanaka & Nagamasu 2006, Tanaka & Hughes 2007, Ito *et al.* 2009, Murata *et al.* 2010, Yukawa *et al.* 2010, Tanaka & Hayami 2011, Ito *et*

*al.* 2014). Here we add three more aquatic monocots to the flora of Myanmar; *Lemna trisulca* L. (Araceae), *Blyxa aubertii* var. *echinosperma* (C.B. Clarke) C.D.K. Cook & Lüönd and *Najas tenuis* A. Braun ex Magnus (Hydrocharitaceae). We also analyzed chloroplast DNA sequences of all three taxa and counted the chromosomes of *N. tenuis* to compare with related taxa and/or other regional materials. The importance and characteristics of these collections are also provided.

### Materials and Methods

Materials of *Lemna trisulca* (Araceae), *Blyxa aubertii* var. *echinosperma*, and *Najas tenuis* (Hydrocharitaceae) were collected on expeditions to Myanmar in 2005 and 2008. The collections were identified using the keys prepared by Cook (1996). The first set of voucher specimens has been deposited in the herbarium of the Forest

Department, Ministry of Environmental Conservation and Forestry, Union of Myanmar (RAF). Duplicates are deposited in the herbaria of the Makino Botanical Garden (MBK) and the University of Tokyo (TI).

Molecular comparisons were made using two slightly different strategies. For *Blyxa aubertii* var. *echinosperma* and *Lemna trisulca*, for which DNA sequences were already available in GenBank, we followed the latest DNA barcoding methods (CBOL Plant Working Group 2009) and chose the chloroplast DNA sequences of *rbcL* and *matK* as molecular markers. For *Najas tenuis*, the more informative *psbA-trnH* was also analyzed in addition to the above two markers, to enable further elucidation of its relationships. The sequences were obtained using the procedure of Ito *et al.* (2010). Each target region was amplified by polymerase chain reaction (PCR) using the following primer pairs: *rbcL* Z1 (Wolf *et al.* 1994) and either *rbcL* 636R (5'-AATAMGAAMCGATCACTCCAACG) modified from Pryer *et al.* (2004) or *rbcL* 1379R (Little & Barrington 2003) for *rbcL*; RM\_749F (Ito *et al.* 2010) and either 8R (Ooi *et al.* 1995) or 1520R (Whitten *et al.* 2000) for *matK*. The obtained sequences were then applied for BLAST search to find comparable sequences (Altschul *et al.* 1990, Altschul *et al.* 1997); those with a 99 % match or closer were obtained for comparison (Tables 1 and 2). Alignments were performed after trimming redundant nucleotides. The final lengths of the alignments were as follows: 606 bp and 542 bp (*rbcL* and *matK* of *B. aubertii*), 1140 bp, 721 bp, and 303 bp (*rbcL*, *matK*, and *psbA-trnH* of *Najas*), and 606 bp and 510 bp (*rbcL* and *matK* of *L. trisulca*), respectively. The sequences obtained in the present study were registered with the DNA Data Bank of Japan (DDBJ), which is linked to GenBank. Their accession numbers are given below (Tables 1 and 2).

For *Najas*, in which polyploidy is widely known (e.g., You *et al.* 1985), we observed the chromosomes of *Najas tenuis* using the procedure of Ito *et al.* (2010).

To determine the geographic range of each species we searched the publications by den Har-

TABLE 1. Nucleotide sequence variation in *rbcL* and *matK* region of chloroplast DNA in *Lemna trisulca*.

Taxon	Accession Number		Locality	<i>matK</i>		
	<i>rbcL</i>	<i>matK</i>		8	247	389
<i>L. trisulca</i>	GU454431	GU454169	Unknown	G	T	T
<i>L. trisulca</i>	AY034237	AY034199	Australia	G	T	T
<i>L. trisulca</i>	GU454429	GU454167	Canada	T	T	G
<i>L. trisulca</i>	HQ901580	N/A	Canada			
<i>L. trisulca</i>	JN892881	JN894635	UK	G	T	T
<i>L. trisulca</i>	JN892881	JN895273	UK	G	G	T
<i>L. trisulca</i>	JN892881	JN895559	UK	G	T	T
<i>L. trisulca</i>	JN892881	JN895561	UK	G	T	T
<i>L. trisulca</i>	GU454430	GU454168	USA	T	T	G
<i>L. trisulca</i>	AB787656*	AB787657*	Myanmar	G	T	T

\*The sequence obtained in the present study.

tog (1957), Dandy (1978), Leach & Osborne (1985), Wang & Sun (1992), Kadono (1994), Cook (1996), Choi (2000), and Hayens (2001).

## Results

### ARACEAE

***Lemna trisulca* L.** in Sp. Pl. 2: 970. 1753. —Fig. 1

*Voucher specimens.* Myanmar, Shan State, Inlay Lake, Nyaung Shwe Township, 20°32'02"N, 96°53'53"E. 3 Dec. 2008, N. Tanaka & al. 080649 (MBK, RAF, TI).

*Distribution.* Bangladesh, N, W, and S China, N and E India, Indonesia (Sumatra, New Guinea), Japan, Malaysia, Myanmar, Papua New Guinea, Pakistan, Philippines; Europe; Oceania; N. America; S. America.

The recovered *rbcL* sequence from the Myanmar collection was identical to sequences from plants in the UK (JN894635, JN895273, JN895559, JN895561), USA (GU454430), Canada (GU454429, HQ901580), Australia (AY034237), and unknown (GU454431). In comparing *matK*, two lineages of *Lemna trisulca* were revealed, one from Canada and the USA and the other from the UK and Australia, plus the unknown (Table 1).

### HYDROCHARITACEAE

***Blyxa aubertii* Rich. var. *echinosperma* (C.B. Clarke) C.D.K. Cook & Lüönd** in Aquat. Bot. 15: 14, f. 3e–h. 1983. —Fig. 2

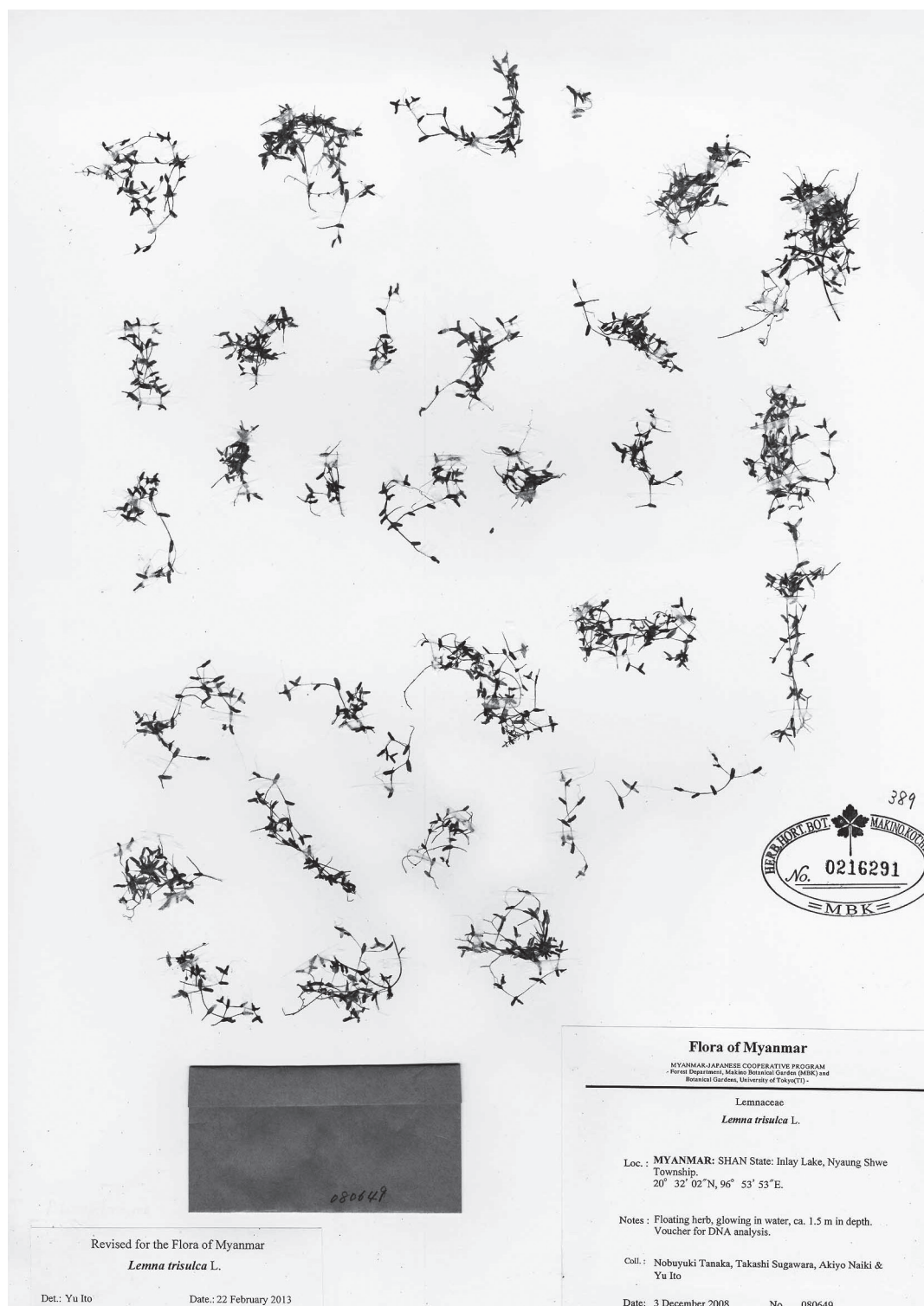


FIG. 1. Voucher specimen of *Lemna trisulca*.





FIG. 2. Voucher specimen of *Blyxa aubertii* var. *echinosperma*.

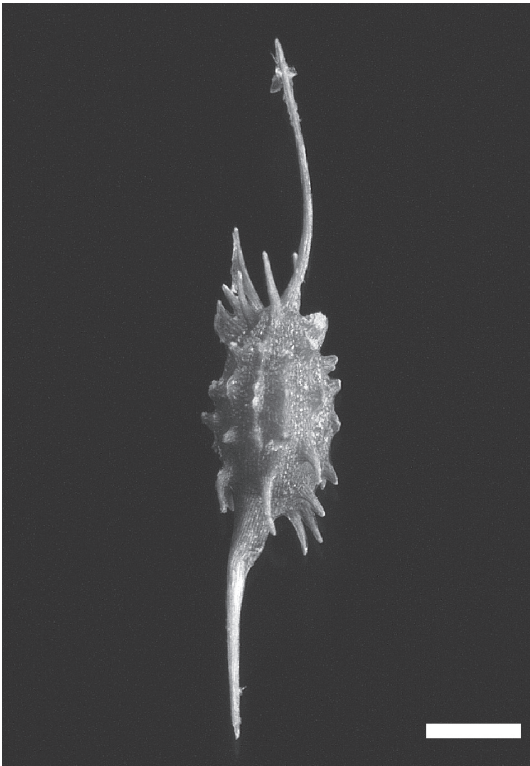


FIG. 3. Fruit morphology of *Blyxa aubertii* var. *echinosperma*. Scale bar = 1 mm.

*Voucher specimen.* Myanmar, Kachin State, 26°40'50"N, 96°15'20"E, in deep lake, 10 Dec. 2005, *J. Murata & al.* 040940 (MBK, RAF, TI).

*Distribution.* Bangladesh, C and S China, W, C, and S India, Indonesia (Java), Japan, Malaysia (Borneo, Peninsular), Myanmar, Nepal, Papua New Guinea, Philippines, Thailand, Sri Lanka; Oceania.

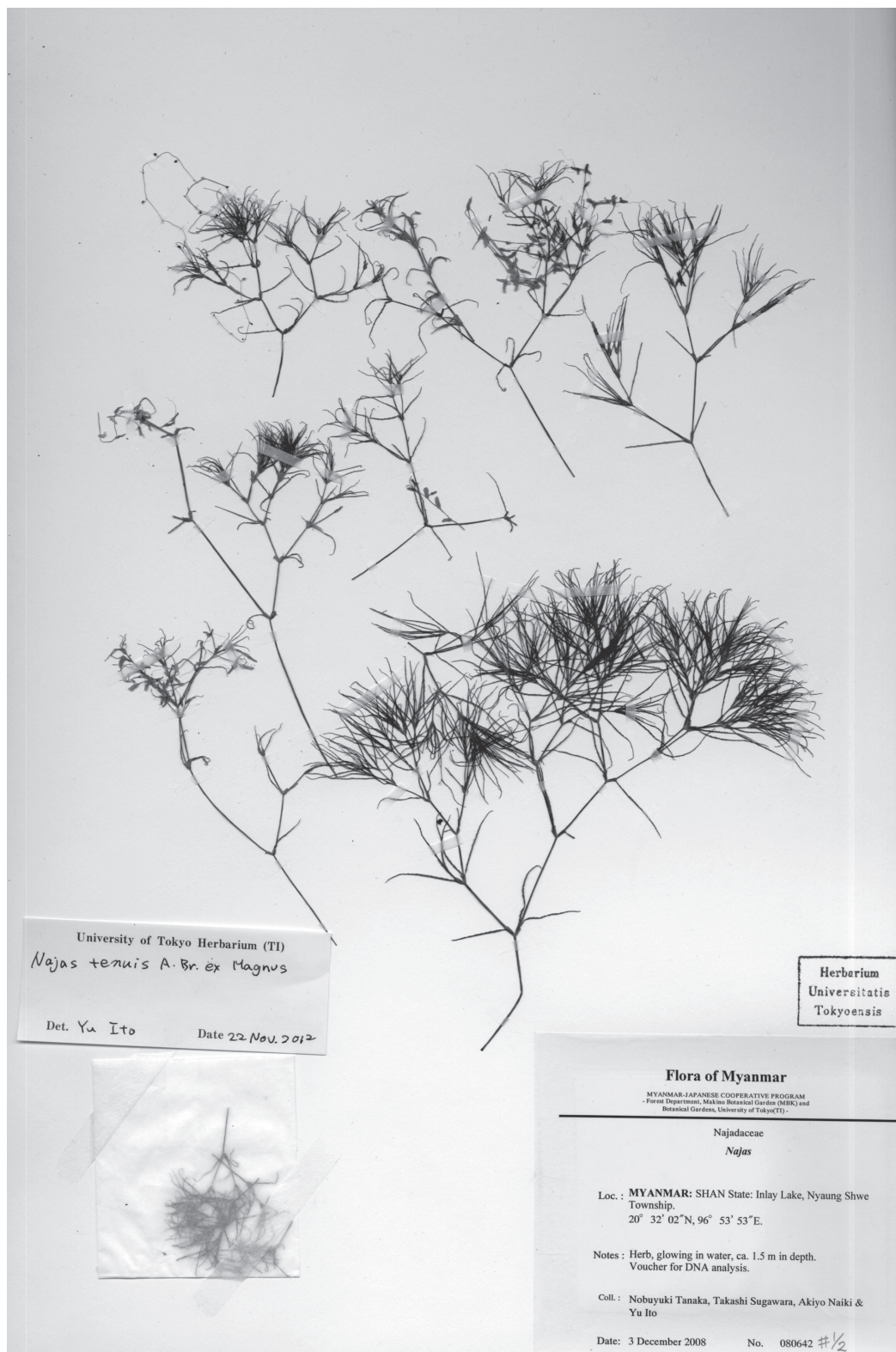
*Blyxa aubertii* var. *echinosperma* is distinguished from var. *aubertii*, also known from Myanmar (Kress *et al.* 2003), by the numerous clear spines on the seed surface and long tails on the edges of the seeds (Fig. 3; Cook & Lüönd 1983). Although the varieties have occasionally been treated as distinct species (e.g., Kadono 1994), given the uniform *rbcL* sequences of *B. aubertii* var. *echinosperma* from Myanmar (AB787654) and Japan (AB088810; including six ambiguous sequence sites) and *B. aubertii* var. *aubertii* from the USA (*rbcL*: U80694), we treat

TABLE 2. Nucleotide sequence variation in *rbcL*, *matK*, and *psbA-trnH* regions of chloroplast DNA in *Najas tenuis* and its closely related species.

Taxon	Accession Number			Locality	<i>rbcL</i>							<i>matK</i>			<i>psbA-trnH</i>					
	<i>rbcL</i>	<i>matK</i>	<i>psbA-trnH</i>		498	513	681	958	1002	1105	77	361	704	96-100	118	134-145	150-167	231	262	274
<i>N. minor</i>	AB004899	AB002579	N/A	Japan	C	T	A	T	T	T	G	C	C			N/A				
<i>N. minor</i>	HM240505	HM240479	N/A	USA	A	T	A	A	T	T	G	C	C			N/A				
<i>N. minor</i>	HM240506	HM240480	N/A	USA	A	T	A	A	T	T	G	C	C			N/A				
<i>Najas</i> sp.	JF975499	JF975502	N/A	China	A	T	A	A	T	C	G	C	-			N/A				
<i>N. minor</i>	N/A	N/A	HQ687184	Korea			N/A	N/A				N/A		AATTT	T	T(11)	GTTGAAAAGA(1)	A	C	C
<i>N. minor</i>	N/A	N/A	HQ687185	Korea			N/A	N/A				N/A		AATTT	T	T(9)	GTTGAAAAGA(1)	A	C	C
<i>N. minor</i>	N/A	N/A	HQ687186	Korea			N/A	N/A				N/A		AATTT	T	T(10)	GTTGAAAAGA(1)	A	G	C
<i>N. minor</i>	N/A	N/A	HQ687187	Korea			N/A	N/A				N/A		----	T	T(11)	GTTGAAAAGA(1)	A	C	C
<i>N. oguraensis</i>	N/A	N/A	HQ687188	Korea			N/A	N/A				N/A		AATTT	T	T(10)	GTTGAAAAGA(1)	A	C	T
<i>N. oguraensis</i>	N/A	N/A	HQ687189	Korea			N/A	N/A				N/A		AATTT	T	T(10)	GTTGAAAAGA(1)	A	C	T
<i>N. oguraensis</i>	N/A	N/A	HQ687190	Korea			N/A	N/A				N/A		AATTT	T	T(11)	GTTGAAAAGA(1)	A	C	C
<i>N. tenuis</i>	AB787658*	AB787659*	AB787660*	Myanmar	A	C	G	A	C	T	A	T	T	AATTT	C	T(12)	GTTGAAAAGA(2)	T	C	C

\*The sequence obtained in the present study.



FIG. 4. Voucher specimen of *Najas tenuis*.

them as varieties. Further genetic comparisons of *matK* revealed no genetic differences between specimens from Myanmar (AB787655) and Japan (AB088781). A morphologically similar species, *Blyxa quadricostata* Hartog, is known from Myanmar and Thailand (Cook 1996, Haynes 2001). The unisexual flowers, spathes with more than 2 flowers, flowers with 6–9 stamens, and obtuse and hooded/boat-shaped leaf tips are unique to *B. quadricostata* (Cook 1996, Haynes 2001).

***Najas tenuis*** A. Braun ex Magnus in Beitr. Kenntn. Najas: 7. 1870. —Fig. 4

*Voucher specimens.* Myanmar, Shan State, Inlay Lake, Nyaung Shwe Township, 20°32' 02"N, 96°53' 53"E. 3 Dec. 2008, *N. Tanaka & al.* 080642 (MBK, RAF, TI).

*Distribution.* C and S India, Myanmar, Sri Lanka.

*Chromosome number.*  $2n = 24$  (Fig. 5)

*Najas tenuis* has been reported from a small portion of tropical Asia, including India and Sri Lanka. A chromosome number,  $2n = 24$ , as well as chloroplast DNA sequence data, implies a

close relationship with *N. minor* (Fig. 5; Table 2;  $2n = 24$ ; China: You *et al.* 1985, You 1989; Russia: Davenport 1980). *N. indica*, under which *N. tenuis* is often synonymized (e.g., Rendle 1899), was determined to be unrelated (97% or less similarity in *psbA-trnH*). The square to hexagonal and somewhat longer than broad seed coat morphology is unique in *N. tenuis* (Fig. 6). In *N. minor* the seed coat is characterized by broader than

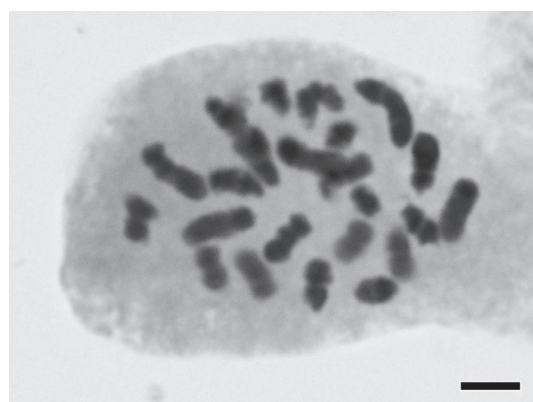


FIG. 5. Somatic chromosomes of *Najas tenuis*. Scale bar = 5  $\mu\text{m}$ .

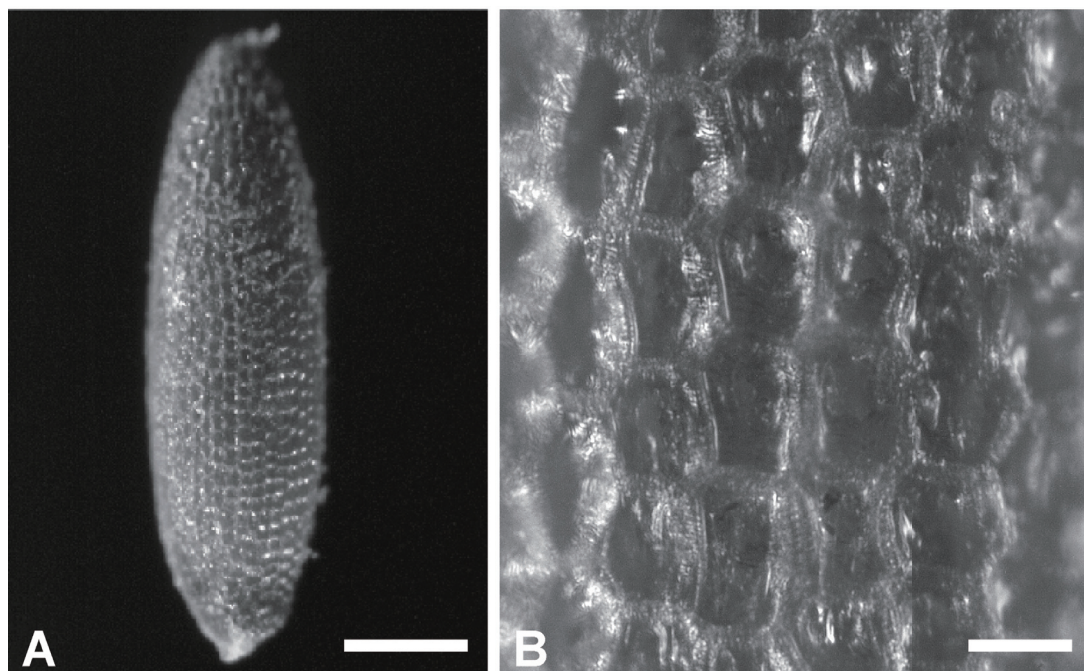


FIG. 6. Seed coat morphology of *Najas tenuis*. A. Whole seed. Scale bar = 0.5 mm; B. Surface pattern of seed coat. Scale bar = 100  $\mu\text{m}$ .



long pits (Cook 1996). Cook (1996) includes SW China in the distribution range of *N. tenuis*, but the Flora of China excludes it (Wang *et al.* 2010).

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